AN IMMUNOHISTOCHEMICAL STUDY OF NEUROTROPHIC FACTORS AND ASSOCIATED CELLS IN THE RAT DENTO-ALVEOLAR COMPLEX SUBJECTED TO ORTHODONTIC FORCES

A thesis submitted in partial fulfilment of the requirement for the degree of Doctor of Clinical Dentistry (Orthodontics)

by

Henry S. H. Ho

Orthodontic Unit
Dental School
Faculty of Health Science
The University of Adelaide
South Australia
AUSTRALIA
2007

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

Ab	Antibody
Anti-NGF	Anti-Nerve growth factor
AP	Activator protein
ABC	Avidin-biotin complex
Ag	Antigen
cAMP	Cyclic adenosine monophosphate
CGRP	Calcitonin gene related peptide
CSF	Colony stimulating factor
DAB	3'-diaminobenzidine tetrahydrochloride
DNA	Deoxyribonucleic acid
DPM2	Distopalatal root of second molar
ECM	Extracellular matrix
EDTA	Ethylenediaminetetra-acetic acid
ERM	Epithelial rests of Malassez
FGF	Fibroblast growth factor
H	Hydrogen
IEG	Immediate early genes
IFN	Interferon
Ig	Immunoglobulin
IGF	Insulin like growth factor
IL	Interleukin
IFN-γ	Interferon gamma
IMVS	Institute of Medical and Veterinary Science
IP3	Inositol triphosphate
IR	Immunoreactive
IU	International units
K	Potassium
LSAB	Labelled streptavidin-biotin
M	Molar (molarity)
M^1	Maxillary first molar
M^2	Maxillary second molar

*m*RNA	Messenger ribonucleic acid
NGF	Nerve growth factor
NGFR	Nerve growth factor receptor
NHS	Normal horse serum
NO	Nitric oxide
NT	Neurotrophin
O.C.T	Optimal cutting temperature
PBS	Phosphate buffered solution
PDL	Periodontal ligament
PDGF	Platelet-derived growth factor
PGE	Prostaglandin E
RNA	Ribonucleic acid
SP	Substance P
TBS	Tris Buffered Solution
TEM	Transmission electron microscopy
TG	Trigeminal ganglion
TGF-β	Transforming growth factor – beta
TNF	Tumour necrosis factor
Trk	Tyrosine receptor kinase
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling
VIP	Vasoactive intestinal polypeptide

Abbreviations of length:

m	Metre
mm	Millimetre
μm	Micrometre
nm	Nanometre

Abbreviations of time:

d	Day
h	Hour
min	Minute
s	Second
wk	Week
y	Year

Abbreviations of volume:

L	Litre
ml	Millilitre
µl	Microlitre

Abbreviations of weight:

g	Gram
kg	Kilogram
mg	Milligram
µg	Microgram
ng	Nanogram
Da	Dalton
kDa	KiloDalton

Research Summary

Biological responses to orthodontic forces involve various cell types, these include fibroblasts, endothelial cells, blood vessels and sensory nerves in the periodontal ligament as well as osteoblasts, osteoclasts and cementoblasts in roots and bone surfaces. Neurotrophins are believed to interact with these cells to initiate the process of bone resorption particularly during orthodontic tooth movement.[1] Neuropeptides released from sensory neurons have been shown to modulate the tissue inflammatory responses.[2, 3] In addition, neurotrophins, including nerve growth factor (NGF), play an important role in neural cell differentiation and survival.[4]

The exact localization and function of neurotrophins and neurotrophic receptors in the dento-alveolar complex remains unclear. Moreover, the identity and distribution of structures expressing neurotrophins and neurotrophic receptors has yet to be fully determined. It is reasonable to propose that periodontal ligament and alveolar bone remodelling may be influenced by NGF. In addition, anti-NGF may block neurochemical changes and, hence, inhibit orthodontic tooth movement.

The aims of this research were to investigate the cells responsible for NGF secretion within the periodontal ligament (PDL), pulp and bone, and the effect that anti-NGF might have on orthodontic tooth movement.

28, 8 week-old, male Sprague-Dawley rats were randomly divided into control and experimental groups. Fourteen experimental animals had anti-NGF injected paradentally. Animals were sacrificed at 7 and 14 days. Sections from an earlier study[5] were examined and stained using TRAP for osteoclast identification and analysed histomorphometrically to enable comparisons between control and experimental groups.

The findings of this investigation indicated that injections of anti-NGF did not significantly affect the rate of tooth movement with the use of different tooth movement measurement methods. TRAP staining proved to be a useful

and reliable marker of osteoclasts. TRAP-positive osteoclastic cells were detected in both anti-NGF and control groups. However, the TRAP-positive cells were not stained intensely with NGF immunolabelling. On the other hand, cells that were stained intensely with NGF, were TRAP-negative. The results suggested that both sympathetic and nociceptive nerves might function in counter balance to modulate bone resorption, and osteoclasts might not be directly responsible for NGF secretion within the PDL and bone.

Further studies to determine the effect of NGF on tooth movement are warranted to more clearly identify the NGF expressing cells within the rat dento-alveolar complex and possible role played by NGF in orthodontic tooth movement.

SIGNED STATEMENT

This report contains no new material that has been accepted for the award of any other degree or diploma in any other university. To the best of my belief, it contains no material previously published except where due reference is made in the text.

I give consent for this copy of my thesis, when deposited in the University library, to be made available for loan and photocopying.

Henry S. H. Ho

Dated:

ACKNOWLEDGEMENTS

I wish to express my appreciation and gratitude to the following people for their invaluable assistance in the completion of this thesis.

Professor Wayne J. Sampson, P.R. Begg Chair in Orthodontics, University of Adelaide, for always finding time from his demanding schedule to offer advice, guidance and editorial assistance.

Dr C. W. Dreyer, Senior Lecturer in Orthodontics, The University of Adelaide, for his time, advice, laboratory assistance and expert opinion.

Dr I. Ferguson, Neurophysiology Department, Flinders University for his time, effort, patience, valuable advice and provision of materials.

Australian Society of Orthodontists for funding this research.

My parents, Patrick and Esther, for their unconditional love, care and support.